

**1473-Pos Board B203****Bilayer Thickness Mismatch Controls Domain Size in Model Membranes**

Frederick A. Heberle<sup>1</sup>, Robin S. Petruzielo<sup>2</sup>, Jianjun Pan<sup>3</sup>, Paul Drazba<sup>4</sup>, Norbert Kucerka<sup>5</sup>, Robert F. Standaert<sup>1,4</sup>, Gerald W. Feigenson<sup>2</sup>, John Katsaras<sup>1,4</sup>.

<sup>1</sup>Oak Ridge National Laboratory, Oak Ridge, TN, USA, <sup>2</sup>Cornell University, Ithaca, NY, USA, <sup>3</sup>University of South Florida, Tampa, FL, USA,

<sup>4</sup>University of Tennessee, Knoxville, TN, USA, <sup>5</sup>Canadian Neutron Beam Centre, Chalk River, ON, Canada.

The spatial organization of lipids and proteins in biological membranes seems to have a functional role in the life of a cell. Diverse evidence supports participation of lipid microdomains (rafts) in membrane processes including protein sorting and signaling. Raft functionality may well involve the reversible coalescence of small and transient domains into larger stable structures that act as platforms for organizing protein machinery. While micrometer-sized domains are observed with some model membrane mixtures, rafts much smaller than 100 nm beyond the reach of optical microscopy are now thought to exist, both *in vitro* and *in vivo*. We have used small-angle neutron scattering, a probe-free technique, to measure the size of nanoscopic membrane domains in unilamellar vesicles. These experiments were performed using a four-component model system containing fixed proportions of cholesterol and the saturated phospholipid 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), mixed with varying amounts of the unsaturated phospholipids 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC). We find that liquid domain size increases with the extent of acyl chain unsaturation (DOPC:POPC ratio). Furthermore, we find a direct correlation between domain size and the mismatch in bilayer thickness of the coexisting liquid-ordered and liquid-disordered phases, suggesting a dominant role for line tension in controlling domain size. While this result is expected from line tension theories, we provide the first experimental verification in free-floating bilayers. Importantly, we also find that changes in bilayer thickness, which accompany changes in the degree of lipid chain unsaturation, are entirely confined to the disordered phase. Together, these results suggest how the size of functional domains in homeothermic cells may be regulated through changes in lipid composition.

**1474-Pos Board B204****Lipid Bilayers Containing Sphingomyelins and Ceramides of Varying N-Acyl Lengths: a Glimpse into Sphingolipid Complexity**

Noemi Jimenez-Rojo, Aritz B. Garcia-Arribas, Jesus Sot, Alicia Alonso, Felix M Goni.

Unidad de Biofisica (CSIC-UPV/EHU), Leioa, Spain.

The thermotropic properties of aqueous dispersions of sphingomyelins (SM) and ceramides (Cer) with N-acyl chains varying from C6:0 to C24:1, either pure or in binary mixtures, have been examined by differential scanning calorimetry. Even in the pure state, Cer and particularly SM exhibited complex endotherms, and their thermal properties did not vary in a predictable way with changes in structure. In some cases, e.g. C18:0 SM, atomic force microscopy revealed coexisting lamellar domains made of a single lipid. Partial chain interdigitation and metastable crystalline states were deemed responsible for the complex behavior. SM: Cer mixtures (90:10 mol ratio) gave rise to bilayers containing separate SM-rich and Cer-rich domains. In vesicles made of more complex mixtures (SM:PE:Chol, 2:1:1), it is known that sphingomyelinase degradation of SM to Cer is accompanied by vesicle aggregation and release of aqueous contents. These vesicles did not reveal observable domain separation by confocal microscopy. Vesicle aggregation occurred at a faster rate for the more fluid bilayers, according to differential scanning calorimetry. Contents efflux rates measured by fluorescence spectroscopy were highest with C18:0 and C18:1 SM, and in general those rates did not vary regularly with other physical properties of SM or Cer. In general the individual SM and Cer appear to have particular thermotropic properties, often unrelated to the changes in N-acyl chain.

**1475-Pos Board B205****Ion Exclusion from Multilamellar Lipid Vesicles**

Johnnie W. Wright, Merrell A. Johnson, Horia I. Petrache.

Department of Physics, Indiana University Purdue University Indianapolis, Indianapolis, IN, USA.

We have shown previously that ions and zwitterionic pH buffers affect the interaction of neutral lipid membranes by a mechanism that involves both a reduction of van der Waals attraction and a creation of electrostatic repulsion. The electrostatic repulsion occurs due to binding of ions and zwitterions to phosphatidylcholine lipid headgroups. To properly analyze lipid interactions, we need to know how salt ions and buffer molecules partition

between lipid bilayers and open solution. To address this issue, we use a sequence of solute concentrations to achieve neutral buoyancy (density matching) for suspensions of dilaurylphosphatidylcholine (DLPC) lipid membranes. We then calculate the ratio of solute concentrations outside and inside the multilamellar lipid vesicles from this density match point. We find that distinct series of monovalent salts, organic salts, and zwitterionic pH buffers are excluded from the interlamellar space, with more polarizable solutes being excluded less, in accord with measurements on membranes interactions. Our quantitative measurements are important for a proper analysis of ionic interactions in membrane systems with applications to membrane biology.

**1476-Pos Board B206****Solution Polarizability Dependence of Lipid Bilayer Interactions**

Merrell A. Johnson<sup>1</sup>, Heather D. Stout<sup>2</sup>, Ryan Z. Lybarger<sup>1</sup>, Horia I. Petrache<sup>1</sup>.

<sup>1</sup>Physics, IUPUI, Indianapolis, IN, USA, <sup>2</sup>Department of Chemistry, Carnegie Mellon University, Pittsburgh, PA, USA.

Neutral lipid bilayers attract each other due to van der Waals (vdW) forces. These forces depend on the electrical polarizability of the aqueous medium in which lipid bilayers are formed. Because attractive vdW forces are balanced by repulsive forces, lipid bilayers form multilamellar vesicles. The equilibrium repeat spacing of multilamellar lipid bilayers is therefore a sensitive and accurate indicator of the force balance between membranes. We present a series of small angle x-ray scattering measurements in which vdW forces are controlled by the nature and electrical polarizabilities of aqueous solutes. We then discuss the interplay of vdW and electrostatic interactions inherent in these systems and highlight their relevance to biological membranes.

**1477-Pos Board B207****Thermodynamic Characterization of the Association of Cholesterol with Phospholipids with Varying Degrees of Unsaturation**

Marshall J. Colville<sup>1,2</sup>, Drake C. Mitchell<sup>1</sup>.

<sup>1</sup>Department of Physics, Portland State University, Portland, OR, USA,

<sup>2</sup>Field of Biophysics, Cornell University, Ithaca, NY, USA.

The preferential association of cholesterol with saturated phospholipids, especially sphingomyelins, is responsible for the formation of coexisting fluid phases in lipid bilayers, and is believed to play a critical role in the existence of more ordered membrane domains in biological systems, sometimes termed lipid rafts. The coexistence of separate fluid phases in model bilayer constructs must be an equilibrium phenomenon, and titration of bilayer cholesterol content allows for thermodynamic characterization of cholesterol partitioning. To achieve a more complete understanding of the parameters governing phase separation, high sensitivity isothermal titration calorimetry was employed to investigate the effects of degree of unsaturation at the *sn*-2 position of a series of biologically relevant phospholipids. Cholesterol was either extracted from or added to bilayers of a single phospholipid component, allowing determination of a partition constant and enthalpy of transfer. By varying the relative cholesterol content of the bilayer, the non-ideal partitioning behavior was also investigated. It was found that the relative affinity of cholesterol for the bilayer decreased in a generally monotonic manner in response to increasing unsaturation, with the exception that both 20:4n6 and 22:6n3 acyl chains at the *sn*-2 position showed similar partition constants and enthalpies of transfer. The partitioning of cholesterol was also measured in membranes composed of mixtures of 18:0,22:6 PC and sphingomyelin. Values of both the partition constant and enthalpy of transfer extrapolated to pure sphingomyelin matched those previously reported for mixtures of 16:0,18:1 PC and sphingomyelin. This result suggests that the association of cholesterol and sphingomyelin is largely unaffected by other phospholipids in the bilayer.

**1478-Pos Board B208****Phase Coexistence in Ternary Lipid Mixtures Containing POPC and Phytosterols, Ergosterol or 7-Dehydrocholesterol**

Mehran Shaghghi<sup>1</sup>, Ming-Yen Kuo<sup>2</sup>, Ya-Wei Hsueh<sup>2</sup>, Martin Zuckermann<sup>1</sup>, Jenifer Thewalt<sup>3</sup>.

<sup>1</sup>Physics, Simon Fraser University, Burnaby, BC, Canada, <sup>2</sup>Physics, National Central University, Jhongli, Taiwan, <sup>3</sup>Molecular Biology & Biochemistry; Physics, Simon Fraser University, Burnaby, BC, Canada.

<sup>2</sup>H-NMR spectroscopy was used to investigate the occurrence of phase coexistence in multilamellar vesicles of DPPC and POPC (33mol%: 33mol%) with either stigmasterol, brassicasterol, ergosterol or 7-DHC (each 33mol %). In all cases, the *sn*-1 chains of DPPC and POPC were deuterated in turn, and <sup>2</sup>H-NMR spectra were measured for both lipid components as a function of temperature between 5 °C and 48 °C. The chain order of DPPC